



HISTOPATHOLOGICAL EVALUATIONS IN SPLEEN AND LIVER OF RATS TREATED WITH METHANOLIC FRUITS EXTRACT OF *CHRYSOPHYLLUM ALBIDUM*

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Article Received on 11/04/2016

Article Revised on 01/05/2016

Article Accepted on 21/05/2016

ABSTRACT

Introduction: Medicinal plants could be used for therapeutic purposes and precursors for the synthesis of useful drugs. The development of novel plant based drugs with enhanced anti-proliferative potential and reduced toxicity has become the prime focus of the evolving medicinal chemistry. In this regards, with over 5000 plants known few are been extensively investigated. In the current study splenic and hepatic toxicity of *Chrysophyllum albidum* was assessed by histopathological evaluation of spleen and liver specimens of rats exposed to the plant. **Methods:** In this research work, 30 healthy male wistar rats weighing between 150-200g were used, with doses of 1000 and 6400 mg/kg of *Chrysophyllum albidum* administered orally for 28 consecutive days using oropharyngeal cannula before sacrificing the animals. After autopsy, the spleen and hepatic tissues were preserved in buffered formalin. Processing of the samples was followed by histopathological evaluation. The results were compared with the normal controls. **Results:** The predominant histopathological finding was mild steatosis, no pathological lesions, normal sinusoids and mild congestion. The hepatic lesions comprised varying extents of moderate vascular congestion and mild infiltration of inflammatory cells. The splenic tissues shows normal red and white pulp, normal spleen capsule. **Conclusion:** The methanolic fruits extract of *Chrysophyllum albidum* causes minimal histological changes in spleen and liver of rats, reflecting its relative safety and use for therapeutic purposes.

KEYWORD: Medicinal plants; spleen; hepatic; *Chrysophyllum albidum*; red pulp; white pulp.

INTRODUCTION

The liver is a vital organ involved in the maintenance of metabolic functions and detoxification of exogenous and endogenous substances.^[1,2] The liver is the key organ maintaining the body homeostasis, involve in various biochemical pathways, fight against diseases, supply nutrients, provide energy and in reproduction.^[3,4] Due to its vital role in drug metabolism, the liver is susceptible to injury from drugs and other substances.^[2]

Spleen considered to be the largest secondary lymphoid organ, contains the body's lymphocytes and it also initiates immune responses to blood-borne antigens^[5,6,7] The spleen consist the red and white pulp. The white pulp is composed of lymphocytes, macrophages, dendritic cells, plasma cells, arterioles, and capillaries in a reticular framework.^[8,9,10] The spleen is actively involved in blood cell productions.^[11] *Chrysophyllum albidum* is a common plant in Southern-western Nigeria widely consumed by both young and old for its nutritional and medicinal values.^[12-17] *Chrysophyllum albidum* plant often grows smaller with the height of 30m from ground level.^[19,20,21] Previous studies showed

that its leaf has antiplatelet and hypoglycemic properties.^[13] Onyeka *et al.*, (2012a) reported that the root bark of *Chrysophyllum albidum* has anti-fertility effects.^[14] The stem bark is used for the treatment of yellow fever and malaria.^[15] Studies have shown that *Chrysophyllum albidum* shown antimicrobial and anti-plasmodial effects.^[16,17] Olorunnisola *et al.*, (2008) reported that the seed cotyledon has been reported to possess anti hyperglycemic and hypolipidemic effects.^[18] *Chrysophyllum albidum* seed cotyledons have been reported to have antimicrobial constituents.^[22]

Inspite of the varieties of the use of *Chrysophyllum albidum* locally and the robust components of this plant there is scarce information on its effect on the spleen as immune modulators. This study was therefore designed to investigate the possible hepatic and immuno effects of the methanolic extract of *Chrysophyllum albidum* fruits on histopathological changes of liver and spleen of wistar rats.

MATERIALS AND METHODS

Plant Material

The fruits of *Chrysophyllum albidum* were collected on 20th January, 2015 from Oyo town in Oyo state, Nigeria and identified by Mr Afilaka, a voucher specimen (FHI 110105) was deposited in Forest Research Institute Nigeria (FRIN). The ripe fruits were washed; the exocarps and mesocarps were manually removed then rinsed in sterile distilled water, cut into small pieces and air-dried.

Plant extraction

The pulverized sample was extracted using methanol in soxhlet apparatus. The extract was collected and evaporated with the aid of a rotatory evaporator.

Experimental animals

All animal were treated in a manner that complied with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (NIH publication, 1985) Federal University of Technology Akure Animal Care approved this research. No specific permission were required for these location, the study was carried out in our department. This study did not involve endangered or protected species.

Healthy male albino rats (n=18) of *Wistar* strain weighing between 150-200g was obtained from the National Veterinary and Research Institute (VOM) were used for this experiment. They were placed in an animal house under standardized laboratory conditions: humidity, temperature (24-25°C) and light 12 h light/dark cycle. They were fed with standard chow (Vital feeds Ltd, Nigeria) and water *ad libitum* throughout the experiment. The animals were exposed to an optimized environment two week before the start of the experiment.

Study design

Eighteen male albino *Wistar* rats were used for the study. The rats were divided into three groups of six rats per group. Group 1 was used as the control group and administered with 2mg/ml corn oil Group 2 and 3 were the treatment groups respectively administered 1000mg/kg and 6400mg/kg body weight of *Chrysophyllum albidum* respectively. The administration was done in 28 days using oropharyngeal cannula. The individual body weights of the animals were evaluated using a top loader weighing balance at the end of each week.

At the end of the administration period, the rats in each group were subjected to overnight fast and subsequently necropsied. During necropsy, liver, spleen, thymus, heart, lungs, stomach, ovaries, uterus, kidney, brain and testes were removed. All organs were visually inspected and weighed directly either after dissection or after fixation to reduce mechanical damage. Spleen and Liver organs were fixed preserved in 10% buffered formalin for further histological processing.

Acute Toxicity Study

Chrysophyllum albidum orally in doses of 1500 mg/kg, 750 mg/kg, 375 mg/kg, 187.5 mg/kg and 93.75 mg/kg, respectively. A control group of 5 animals was simultaneously administered 0.2 ml water orally. After 48 hours, the number of deceased rats was counted in each group and LD50 (dose that kills 50% of animals) was calculated (322 mg/kg) by the Organisation for Economic Co-operation and Development method OECD (2008) Assessment No 19.^[23]

Histopathology

a) Tissue processing

The formalin preserved splenic and hepatic tissue samples of *Chrysophyllum albidum* dosed rats and controls were processed according to method of Avwioro (2010). The tissues were grossed first, observed and cut into small pieces of not more than 4mm thick into pre-labelled cassettes. These were further immersed in 10% formol saline for 24 hours to fix. Followed by tissue processing; this is done automatically using automatic tissue processor (Leica tp1020). The tissue were allowed to pass through various reagents including; station one containing 105 formol saline, station 3 to station 7; alcohol (70%, 80%, 90%, 95%, absolute 1 & absolute 11) for the purpose of dehydration. The tissues continued to pass through station 8 and station 9 containing two changes of xylene for the purpose of clearing and finally transferred into three wax baths for infiltration/impregnation. The machine has been programmed to run for 12 hours, tissues stayed in each station for 1hour. After processing the tissues, the next stage is embedding; each processed tissue was given a solid support medium (paraffin wax) and this is done using a semi-automatic tissue embedding center. The molten paraffin wax was dispensed into a metal mold and the tissue was buried and oriented in it, a pre labelled cassettes was placed on this and are transfered to a cold plate to solidified. The tissue block formed was separated from the mold. The next step is microtomy: the blocks were trimmed to expose the tissue surface using a rotary microtome at 6 micrometer. The surfaces were allowed to on ice before sectioning. The tissues were sectioned at 4 micrometer (ribbon section). The sections were floated on water bath set at 55^oc and these were picked using clean slides. The slides were labelled. The slides are dried on a hotplate (raymonlamb) set at 60^oc for 1hour. The staining technique used is haematoxylin and eosin technique.

b.) Procedure for Haematoxylin and Eosin (H&E) Technique

Dewax in Xylene for 15mins, Take through Absolute Alcohol, 95% and 70% Alcohol, Rinse the section to water. Stain in Harris haematoxylin for 5mins. Rinse in water, Differentiate in 1% acid alcohol briefly. Rinse in water. Blue under running tap water for 10mins, Counterstain with 1% aqueous Eosin for 2min. Rinse in water. Dehydrate in ascending grades of alcohol. Clear in xylene and Mount in DPX. Stained sections were

examined for necrosis, apoptosis, inflammation and vascular changes in renal tissue. The hepatic tissue was evaluated for any alterations in the architecture, portal or lobular inflammation, sinusoidal dilatation and congestion along with presence of granulomas, degeneration, necrosis and fatty change.

c.) Histopathological categorization of hepatic lesions. The hepatic lesions were categorized according to the criteria mentioned below by Ramchandran *et al*^[24] “Table 1”.

Table 1: Hepatic microscopic findings of methanolic extract of *Chrysophyllum albidum* fruits.

GROUPS	CONGESTION		SINUSOIDS			INFLAMMATORY CELLS	HEPATOCELLULAR NECROSIS	
	Mild	Marked	Mild	Mod	Marked		Cell degeneration	Necrosis
A	20%	---	20%	---	----	Without inflammation	----	---
B	20%	----	20%	----	----	Without inflammation	20%	-----
C	40%	-----	40%	-----	-----	Without inflammation	-----	20%

RESULTS

Acute toxicity test

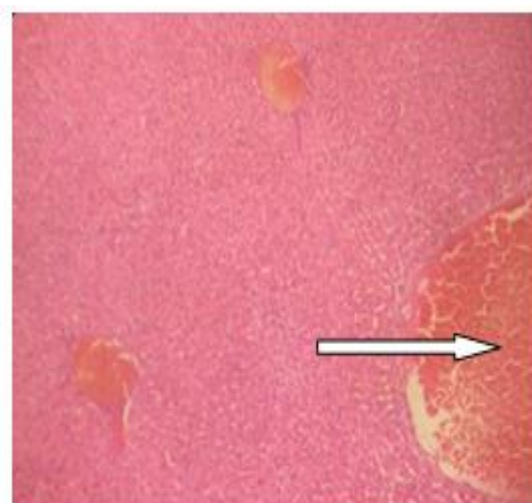
Acute toxicity test did not show any physical signs and symptoms of lethality even at the highest dose of 5000mg/kg, an indication that the extract is non toxic with a wide safety margin when it is ingested orally.

28 days study of splenic microscopic findings.

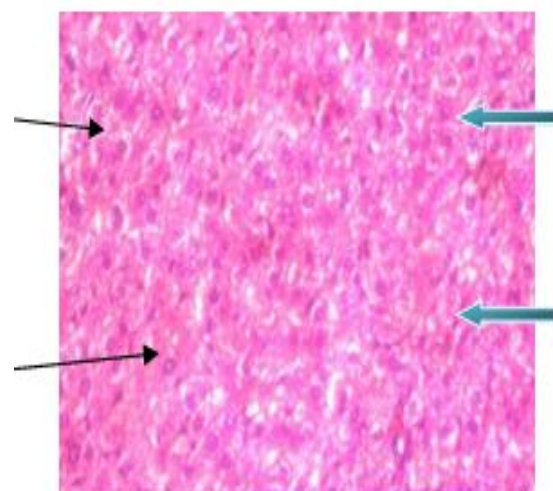
In the entire groups (1-3) used for this study no specific necrosis grade was evident. The spleen microscopy in Group 1 (control group) showed normal splenic tissue, no pathological lesion seen. The red pulp (blue arrow) and white pulp (white arrow) seen appear normal, the spleen capsule (black arrow) appears normal. “Figure 2” In group 2 (Dose: 1000 mg/kg), all the animals survived before sacrificing. Spleen microscopy showed mild vascular congestion and mild haemorrhage (black arrow), normal splenic tissue, the red pulp (blue arrow) and white pulp (white arrow) seen appear normal “Figure 4” In Group 3 (Dose: 6400 mg/kg body weight) the spleen histology was within normal limits “Figure 6” showing moderate vascular congestion (black arrow), the red pulp (blue arrow) and white pulp (white arrow) seen appear normal.

28 days study of hepatic microscopic findings

The hepatic lesion comprised normal architecture to mild infiltration and moderate congestion “Figure 1” with one case revealing that the liver parenchyma showed focal area of mild lymphocytes aggregate. Group 1 revealed that central venules appear mildly congested (white arrow), the sinusoids appear normal (slender arrow) without infiltration of inflammatory cells. the hepatocytes show normal morphology (blue arrow). “Figure 1” In Group 2 the liver histology was within normal limits “Figure 3” with the sinusoids appear normal (slender arrow) without infiltration of inflammatory cells. the hepatocytes show vessicular nuclei (blue arrow). The hepatic microscopy in Group 3 showed moderate vascular congestion seen (white arrow), the sinusoids with mild infiltration of inflammatory cells (slender arrow) and normal morphology of hepatocytes (blue arrow) “Figure 5”. The hepatic picture ranged from normal, unaffected liver in the three groups.

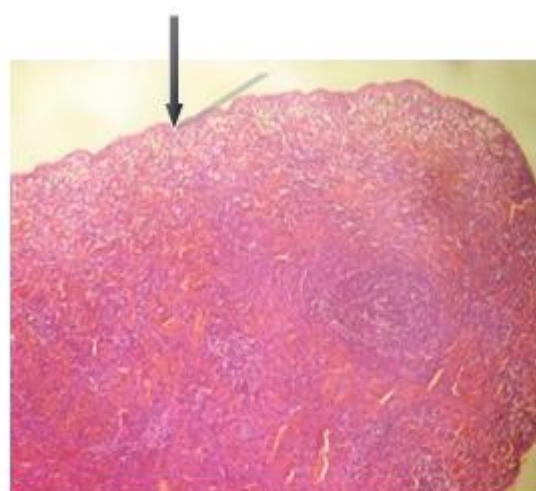


(a)

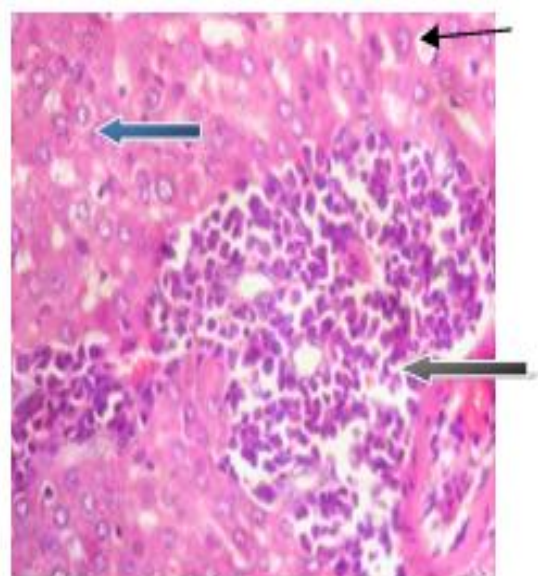


(b)

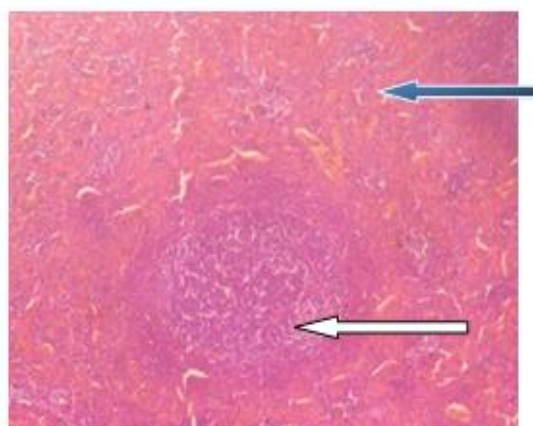
Figure 1: Photomicrograph of liver section in the control group. (a) showing normal architecture as seen in lower magnification (H&E x100), (b) showing central venules which appear mildly congested (white arrow), the sinusoids appear normal (slender arrow) without infiltration of inflammatory cells. The hepatocytes show normal morphology (blue arrow) (H&E x400). Nuclei – blue, Cytoplasm – pink



(a)



(b)

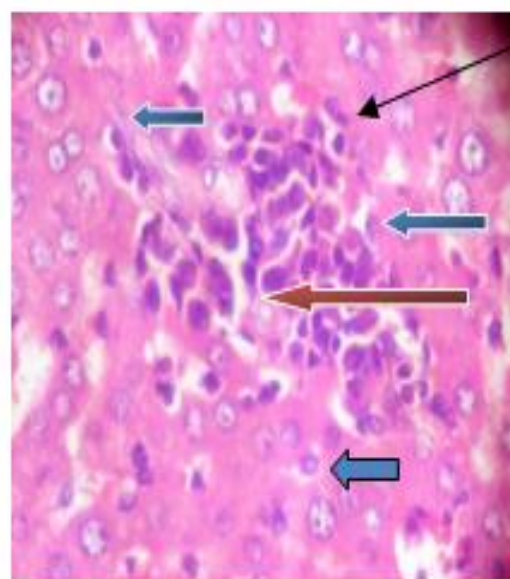


(b)

Figure 2: Photomicrographs of a splenic section of the control group stained by haematoxylin and eosin. (a) showing normal splenic tissue, no pathological lesion seen. The spleen capsule (black arrow) appears normal. (H&Ex100) (b) showing normal splenic tissue, the red pulp (blue arrow) and white pulp (white arrow) seen appear normal (H&E x400).

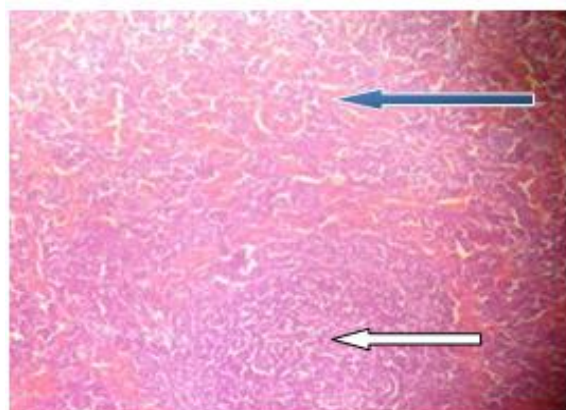


(a)

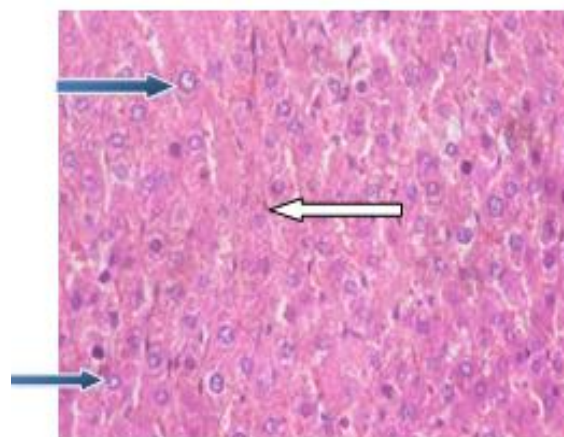


(c)

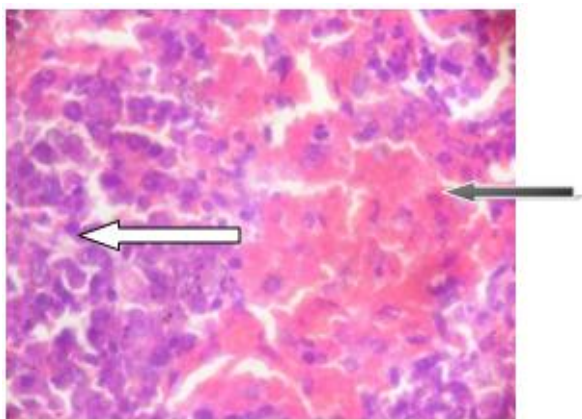
Figure 3: Photomicrograph of liver section of group treated with 1000 mg/kg body weight of methanolic extract of *Chrysophyllum albidum* fruits. (a) showing normal architecture as seen in lower magnification (H&E x100), (b) the central venules show mild congestion (white arrow), mild perivascular infiltration of inflammatory cells (black arrow), The hepatocytes show vesicular nuclei (blue arrow), the sinusoids appear normal (slender arrow) (H&E X400). (c) the liver parenchyma shows focal area of mild lymphocytes aggregate (red arrow). The sinusoids appear normal (slender arrow) without infiltration of inflammatory cells. The hepatocytes show vesicular nuclei (blue arrow). (H&E X400).



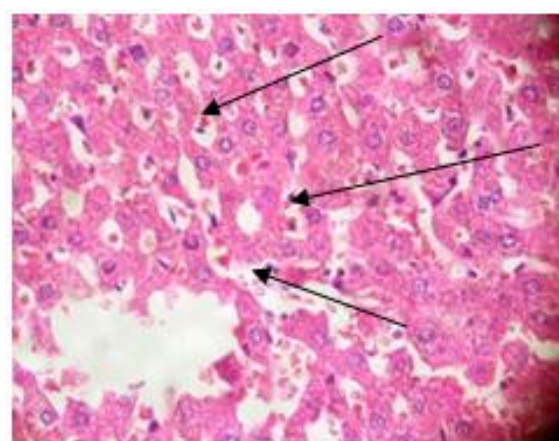
(a)



(b)



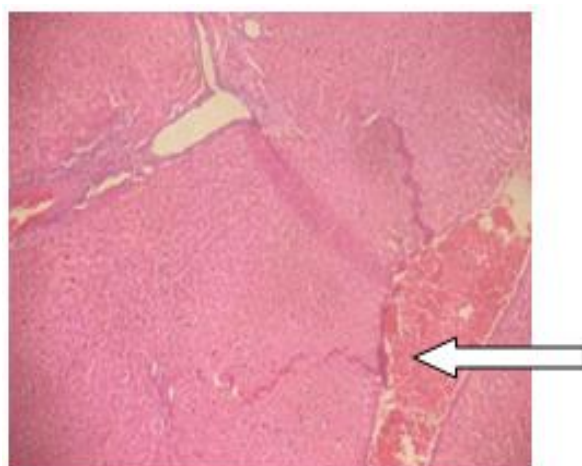
(b)



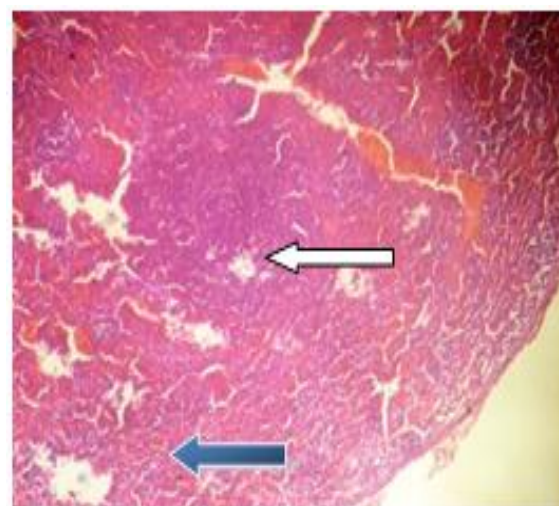
(c)

Figure 4: Photomicrographs of a splenic section of rats treated with 1000 mg/kg body weight of methanolic extract of *Chrysophyllum albidum* fruits stained by haematoxylin and eosin (a) showing normal splenic tissue, the red pulp (blue arrow) and white pulp (white arrow) seen appear normal. (H&E x100) (b) showing mild vascular congestion and mild haemorrhage (black arrow), normal splenic tissue and white pulp (white arrow) seen appear normal. (H&E x400).

Figure 5: Photomicrograph of liver section treated with 6400 mg/kg body weight of methanolic extract of *Chrysophyllum albidum* fruits (a) showing mild vascular congestion (white arrow). (H&E x100) (b) the hepatocytes show normal morphology (blue arrow) and mild vascular congestion (white arrow). (H&E x400) (c) the sinusoids show mild infiltration of inflammatory cells (slender arrow). (H&E x400).



(a)



(a)

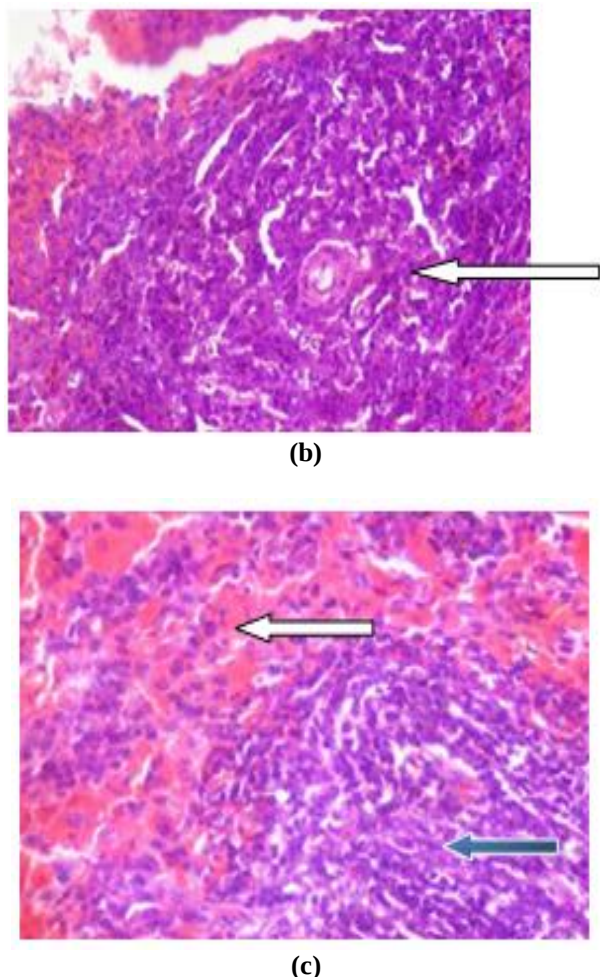


Figure 6: Photomicrographs of a splenic section treated with 6400 mg/kg body weight of methanolic extract of *Chrysophyllum albidum* fruits showing (a) the red pulp (blue arrow) and white pulp (white arrow) seen appear normal (H&E x100) (b) white pulp (white arrow) seen appear normal (H&E x40) (c) the red pulp (blue arrow) and white pulp (white arrow) seen appear normal (H&E x 400).

DISCUSSION

The active ingredients of plants have provided sources of compounds in the development of new therapeutics.^[25] Some naturally occurring active ingredients from plants have antioxidant properties.^[26-30] These naturally occurring plants have potential to be hepatoprotective and immunoprotective and therefore can be considered for use in the treatment of acute and chronic diseases. Our findings demonstrated hepatic and immune effects of *Chrysophyllum albidum*. The methanolic extract of *Chrysophyllum albidum* fruits showed much extended splenic and hepatic safety range. Acute toxicity test of the methanolic extract of *Chrysophyllum albidum* fruits did not show any physical signs and symptoms of lethality, an indication that the extract is non toxic with a wide safety margin when ingested orally. Comparative analysis of spleen and liver with *Chrysophyllum albidum* was limited in recent studies. Previous studies showed that *Chrysophyllum albidum* contains tannins,

flavonoids, saponins, alkaloids, anthraquinone and cardenolides.^[16,17,41] Many studies report *Chrysophyllum albidum* having, antimicrobial, anti-fertility effects and anti-plasmodial effects^[16,17,14] with elaborated antiplatelet, hyperglycemic, hypolipidemic and hypoglycemic properties^[13,18,31,32] invivo and invitro in rats and mice, but their protective data as regards detailed splenic and hepatic histopathological manifestations have not been studied. Liver is the most important site of xenobiotic metabolism, and if damage can lead to deleterious effect on liver functions.^[33] Liver cells acquire natural antioxidants such as superoxide dismutase, catalase, glutathione peroxidase and glutathione. Antioxidants are capable of scavenging free radicals by inhibiting oxidation by quenching of triplet oxygen.^[34-37] Recently, there has been a great deal of interest in edible plants, fruits and vegetables that hold antioxidants and phytochemicals.^[38] In our present study, the hepatic lesion comprised normal architecture to mild infiltration and moderate congestion (Figure 1 and 3) with one case revealing that the liver parenchyma showed focal area of mild lymphocytes aggregate.(Figure 5) The spleen is the site of hematopoiesis which contains vascular and lymphoid elements.^[6,39-40] The spleen in some species is involved in the removal of effete such as degenerate and aged red blood cells, particulate materials from the blood supply. The spleen is one of the important components of the immune system with the red pulp and the white pulp as its substances. In this research work, 28 days microscopic study revealed normal splenic tissue, no pathological lesion seen. The red pulp (blue arrow) and white pulp (white arrow) seen appear normal, the spleen capsule (black arrow) appears normal. (Figure 2) in the control group, In treatment group (Dose: 1000 mg/kg), all the animals survived before sacrificing, normal splenic tissue, the red pulp (blue arrow) and white pulp (white arrow) seen appear normal (Figure 4) also at Dose: 6400 mg/kg body weight groups the spleen histology was within normal limits (Figure 6) showing moderate vascular congestion (black arrow), the red pulp (blue arrow) and white pulp (white arrow) seen appear normal.

CONCLUSIONS

It can be concluded that the methanolic extract of *Chrysophyllum albidum* fruits showed much extended splenic and hepatic safety range. In the groups of animals used for this study, no hepatic necrosis was seen also no evidence of hepatocytic degeneration. The histopathological changes observed in the spleen may increase immunological competency and increased hematopoiesis due to *Chrysophyllum albidum* fruits. This research will be useful for folks both young and old in all parts of Nigeria that consume *Chrysophyllum albidum* for its nutritional and medicinal values.

ACKNOWLEDGEMENTS

We acknowledge the services of Mr Peter Otegbade for the technical assistances and Mr Afilaka for authentication of the plant material.

Conflict of Interest

All authors declare that there is no conflict of interest for this research work.

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